DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

July 28, 2015

Alere Scarborough, Inc.
Angela Drysdale
Vice President, Regulatory and Clinical Affairs – Infectious Disease
10 Southgate Road
Scarborough, ME 04074

Re: K151464
Trade/Device Name: Alere™ i Influenza A&B
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: II
Product Code: OCC, OZE, OOI
Dated: June 02, 2015
Received: June 03, 2015

Dear Ms. Drysdale:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Tamara V. Feldblyum -S for

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K151464

Device Name
Alere™ i Influenza A & B

Indications for Use *(Describe)*
The Alere™ i Influenza A & B assay performed on the Alere™ i Instrument is a rapid molecular in vitro diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2012-2013 and the 2014-2015 influenza seasons when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(K) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is: K151464

SUBMITTER
Alere Scarborough, Inc.
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Scarborough, ME 04074
Establishment Registration Number: 1221359

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DATE PREPARED
7/8/2015

TRADE NAME
Alere™ i Influenza A & B

COMMON NAME
Alere™ i flu, Alere™ i, Alere™ Influenza A & B

CLASSIFICATION NAME
Respiratory Viral Panel Multiplex Nucleic Acid Assay (per 21 CFR 866.3980)
Instrumentation for Clinical Multiplex Test Systems (per 21 CFR 862.2570)

CLASSIFICATION
Class II

PRODUCT CODE
OCC, OZE, OOI

PANEL
Microbiology (83)

PREDICATE DEVICE
Alere™ i Influenza A & B Assay, K141520

DEVICE DESCRIPTION

Alere™ i Influenza A & B is a rapid, instrument-based isothermal test for the qualitative detection and differentiation of influenza A and influenza B from nasal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients presenting with signs and symptoms of respiratory infection. The Alere™ i Influenza A & B System utilizes isothermal nucleic acid amplification technology and is comprised of:

- Sample Receiver – single use, disposable containing the elution buffer
- Test Base – single use, disposable comprising two sealed reaction tubes, each containing a lyophilized pellet
- Transfer Cartridge – single use, disposable for transfer of the eluted sample to the Test Base, and
- Alere™ i Instrument – repeat use reader

The reaction tubes in the Test Base contain the reagents required for amplification of the target nucleic acid and an internal control. Alere™ i Influenza A & B utilizes a pair of templates (similar to primers) for the specific amplification of RNA from influenza A and B and a fluorescently labeled molecular beacon designed to specifically identify the amplified RNA targets. Alere™ i Influenza A & B is performed within the confinement of the Test Base, and no other part of the Alere™ i Instrument has contact with the sample during the amplification process. This minimizes the risk of instrument contamination and sample carry-over between measurements.

To perform the assay, the Sample Receiver and Test Base are inserted into the Alere™ i Instrument and the elution buffer is automatically heated by the instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, resuspending the lyophilized pellet contained within the Test Base and initiating target amplification. Heating, mixing and detection by fluorescence is provided by the instrument, with results automatically reported.

Results are displayed by the Alere™ i Instrument separately for influenza A and influenza B. Results are also stored in an on-board archive and are assigned to a sample ID that has been entered into the Alere™ i Instrument by the operator, and the date/time the test was performed. Data can be retrieved and downloaded by the operator at any time after testing. An external Alere™ Universal Printer can be attached via USB to the Alere™ i Instrument to print test results.

INTENDED USE

The Alere™ i Influenza A & B assay performed on the Alere™ i Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2012-2013 and the 2014-2015 influenza seasons when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

TECHNOLOGICAL CHARACTERISTICS

The purpose of this submission is to expand the Alere™ i Influenza A & B test Intended Use (hereby referred to as Expanded Claim Alere™ i Influenza A & B test) to include testing of swab samples that have been eluted in viral transport media. There have been no changes to the Alere™ i Influenza A & B test or principles of operation.

DEVICE COMPARISON

The expanded claim Alere™ i Influenza A & B test was compared to the legally marketed predicate device, the 510(k) cleared Alere™ i Influenza A & B test.

Parameter	**Expanded Claim Alere™ i Influenza A & B**	**Alere™ i Influenza A & B (K141520)**
FDA Product Code	OCC,OZE, OOI	Same
Assay Target	Influenza A, Influenza B	Same
Intended Use	The Alere™ i Influenza A & B assay performed on the Alere™ i Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in nasal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus. Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Performance characteristics for influenza A were established during the 2012-2013 influenza season when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral	The Alere™ i Influenza A & B assay performed on the Alere™ i Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection and discrimination of influenza A and B viral RNA in nasal swab samples from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus. Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Performance characteristics for influenza A were established during the 2012-2013 influenza season when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Parameter	Expanded Claim Alere™ i Influenza A & B	Alere™ i Influenza A & B (K141520)
	culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	
Intended Environment for Use	CLIA waived for testing direct nasal swab samples only. Professional use, in a medical laboratory or point-of-care.	CLIA Waived. Professional use, in a medical laboratory or point-of-care.
Instrumentation	Alere™ i Instrument	Same
Self-Contained System	Integrated PC, Software, and Touch Screen Display	Same
Automated Assay	Yes. Sample preparation, amplification, detection, and result interpretation.	Same
Assay Information		
Sample Type	Nasal Swab and Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media	Nasal Swab
Influenza A Viral Target	PB2 segment	Same
Influenza B Viral Target	PA segment	Same
Technology	Isothermal nucleic acid amplification for detecting the presence/absence of viral RNA in clinical specimens	Same
Detection Method	Assay uses different reporter dyes for each target	Same
Internal Control	Yes	Same
Result Interpretation	Automated	Same
Assay Result	Qualitative	Same
Time to Result	< 15 minutes	Same

SUBSTANTIAL EQUIVALENCE

Expansion of the Alere™ i Influenza A & B Intended Use did not result in significant changes to the Analytical Performance of the test.

PERFORMANCE SUMMARY

ANALYTICAL STUDIES

ANALYTICAL SENSITIVITY

Alere™ i Influenza A & B limit of detection (LOD) in natural nasal swab matrix was determined by evaluating different concentrations of 3 strains of influenza A and 2 strains of influenza B virus in Alere™ i Influenza A & B. Three strains of influenza A virus representing each of the three common currently or recently circulating influenza A subtypes (i.e., A/H1N1, A/H3N2 seasonal, and A/H1N1 pandemic (pdm)) and two strains of influenza B virus representing each of the two influenza B genetic lineages (i.e., Victoria and Yamagata) were included in this study.

Presumed negative natural nasal swab specimens were eluted in UTM. Swab elutes were combined and mixed thoroughly to create a clinical matrix pool to be used as the diluent. Each influenza virus strain was diluted in this natural nasal swab matrix pool to generate virus dilutions for testing. The vender provided virus strains were re-titered and the concentrations (in $TCID_{50}$/mL) were determined by standard virologic method. The concentration for each dilution (in genome equivalents/mL) was also assessed using laboratory developed and validated influenza A and influenza B quantitative real-time PCR assays.

Contrived nasal swab samples were prepared by coating 10 microliters of each virus dilution onto the swab. The contrived swab samples were tested without further elution in viral transport media according to the test procedure for Direct Nasal Swab.

An additional LOD study was conducted with contrived swab samples eluted into VTM and tested according to the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media.

The LOD for each influenza strain tested was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive).

The confirmed LODs in natural nasal swab matrix for both direct swab and swab eluted in VTM for each influenza strain tested are presented in the tables below:

Limit of Detection (LOD) Study Results – Natural Nasal Swab Matrix (Direct Swab Testing)

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	LOD ($TCID_{50}$/mL)	LOD ($TCID_{50}$/Swab)*	LOD (Genome Equivalents/mL)	LOD (Genome Equivalents/Swab)*
A/Puerto Rico/8/34	A/H1N1	1.88×10^5	1.88×10^3	4.22×10^6	4.22×10^4
A/Perth/16/2009	A/H3N2	8.60×10^2	8.60×10^0	7.91×10^4	7.91×10^2
A/California/7/2009	A/2009 H1N1 pdm	1.25×10^4	1.25×10^2	5.20×10^6	5.20×10^4
B/Malaysia/2506/2004	B Victoria lineage	1.90×10^3	1.90×10^1	7.24×10^4	7.24×10^2
B/Bangladesh/3333/2007	B Yamagata lineage	5.55×10^2	5.55×10^0	7.36×10^4	7.36×10^2

*Note: 10 ul of each virus dilution was coated onto a swab

Limit of Detection (LOD) Study Results – Natural Nasal Swab Matrix (Swab Eluted in VTM Testing)

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	LOD ($TCID_{50}$/mL)	LOD ($TCID_{50}$/Swab)*	LOD (Genome Equivalents/mL)	LOD (Genome Equivalents/Swab)*
A/Puerto Rico/8/34	A/H1N1	4.20×10^5	4.20×10^3	4.59×10^6	4.59×10^4
A/Perth/16/2009	A/H3N2	9.82×10^3	9.82×10^1	1.25×10^6	1.25×10^4
A/California/7/2009	A/2009 H1N1 pdm	5.20×10^5	5.20×10^3	7.77×10^6	7.77×10^4
B/Malaysia/2506/2004	B Victoria lineage	1.05×10^5	1.05×10^3	2.29×10^6	2.29×10^4
B/Bangladesh/3333/2007	B Yamagata lineage	1.34×10^4	1.34×10^2	1.98×10^6	1.98×10^4

*Note: 10 ul of each virus dilution was coated onto a swab; each contrived swab was further diluted into 3 mL of UTM

REACTIVITY TESTING

An analytical reactivity (inclusivity) study was performed to determine whether the Alere™ i Influenza A & B assay is able to detect a variety of influenza A and B strains that represent temporal and geographic diversity.

Vender provided stocks of influenza A and B strains were diluted in UTM to generate virus dilutions for testing. The concentration (in $TCID_{50}$/mL, $CEID_{50}$/mL, or EID_{50}/mL) for each strain was determined by standard virologic method. The concentration for each dilution (in genome equivalents/mL) was also assessed using laboratory developed and validated influenza A and influenza B quantitative real-time PCR assays.

Contrived swab samples were prepared by coating 10 microliters of virus dilution onto each swab. The contrived swab samples were tested without further elution in viral transport media according to the test procedure for Direct Nasal Swab.

The starting dilution concentration selected for testing in this study was higher than the established LODs in the Limit of Detection study. Each starting dilution per virus strain was tested in triplicates initially. If the initial testing concentration tested positive for all three replicates, the strain was further diluted 10-fold and tested in triplicates until at least one out three replicates generated a negative result. When a negative result was obtained, additional 2-fold dilutions were tested, starting from the highest dilution that produced 100% (3/3) positive results. A concentration level was considered "reactive/positive" in this study for all but one strain tested (i.e., B/Texas/06/2011 – see footnote "c" under the table below) if all three replicates generated a positive result for the expected influenza virus.

The Alere™ i Influenza A & B assay detected all strains tested at the concentrations indicated in the table below:

Analytical Reactivity Study Results

Influenza Strain	Influenza A Subtype or Influenza B Genetic Lineage	Test Concentration (in $TCID_{50}$ or Genome Equivalents , unless indicated otherwise)				Flu A Result (n=3, unless indicate otherwise)	Flu B Result (n=3, unless indicate otherwise)
		$TCID_{50}$/mL	$TCID_{50}$/Swab*	Genome Equivalents/ mL	Genome Equivalents/Swab*		
A/New Caledonia/20/1999[a]	A/H1N1	9.19×10^5	9.19×10^3	4.09×10^6	4.09×10^4	+	-
A/New Jersey/8/76[a]	A/H1N1	3.41×10^1	3.41×10^{-1}	1.52×10^5	1.52×10^3	+	-
A/Brisbane/59/2007[a]	A/H1N1	2.11×10^4	2.11×10^2	3.39×10^5	3.39×10^3	+	-
A/WSN/33[a]	A/H1N1	2.11×10^2	2.11×10^0	2.43×10^5	2.43×10^3	+	-
A/Port Chalmers/1/73	A/H3N2	4.22×10^4	4.22×10^2	1.31×10^6	1.31×10^4	+	-
A/Hong Kong/8/68	A/H3N2	7.03×10^0	7.03×10^{-2}	2.70×10^5	2.70×10^3	+	-
A/Aichi/2/68	A/H3N2	2.08×10^5	2.08×10^3	7.47×10^6	7.47×10^4	+	-
A/Victoria/3/75	A/H3N2	3.68×10^5	3.68×10^3	3.39×10^6	3.39×10^4	+	-
A/Wisconsin/67/2005	A/H3N2	6.81×10^4	6.81×10^2	2.57×10^6	2.57×10^4	+	-
A/Brisbane/10/2007	A/H3N2	3.16×10^2	3.16×10^0	3.37×10^5	3.37×10^3	+	-
A/Texas/50/2012	A/H3N2	2.5×10^0	2.50×10^{-2}	6.35×10^3	6.35×10^1	+	-
A/Victoria/361/2011	A/H3N2	1.56×10^1	1.56×10^{-1}	3.53×10^5	3.53×10^3	+	-
A/California/4/2009	A/H1N1 (pdm)	1.47×10^4	1.47×10^2	1.07×10^6	1.07×10^4	+	-
A/Maryland/04/2011	A/H1N1 (pdm)	7.88×10^4	7.88×10^2	3.81×10^6	3.81×10^4	+	-
A/New York/18/2009	A/H1N1 (pdm)	1.25×10^2	1.25×10^0	9.16×10^5	9.16×10^3	+	-
A/Anhui/1/2013 (Inactivated)[a]	A/H7N9 (Detected in China in 2013)	4.00×10^6 EID_{50}/mL	4.00×10^4 EID_{50}/Swab	1.72×10^6	1.72×10^4	+	-
A/Indiana/10/2011[a]	A/H3N2v	2.00×10^8 EID_{50}/mL	2.00×10^6 EID_{50}/Swab	5.94×10^4	5.94×10^2	+	-
B/Lee/40	Victoria Lineage	5.00×10^1 $CEID_{50}$/mL	5.00×10^{-1} $CEID_{50}$/Swab	5.40×10^4	5.40×10^2	-	+
B/Victoria/504/2000	Victoria Lineage	1.19×10^3	1.19×10^1	6.24×10^4	6.24×10^2	-	+
B/Nevada/03/2011	Victoria Lineage	1.75×10^3	1.75×10^1	8.29×10^4	8.29×10^2	-	+
B/Montana/05/2012	Victoria Lineage	9.00×10^1	9.00×10^{-1}	2.55×10^4	2.55×10^2	-	+
B/Maryland/1/59	Yamagata Lineage	8.51×10^2	8.51×10^0	1.13×10^5	1.13×10^3	-	+
B/Russia/69[b]	Yamagata Lineage	4.44×10^1	4.44×10^{-1}	2.96×10^6	2.96×10^4	-	+
B/Wisconsin/01/2010[c]	Yamagata Lineage	3.68×10^4	3.68×10^2	1.16×10^6	1.16×10^4	-	+
B/Massachusetts/2/2012	Yamagata Lineage	6.25×10^1	6.25×10^{-1}	2.28×10^5	2.28×10^3	-	+
B/Texas/06/2011[c]	Yamagata Lineage	2.89×10^5	6.25×10^3	2.00×10^6	2.00×10^4	-	+

*Note: 10 ul of each virus dilution was coated onto a swab

[a] Although this test has been shown to detect A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/H3N2v viruses cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for the A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/H3N2v viruses have not been established.

[b] Influenza B/Russia/69 lowest level in which 3/3 replicates were positive is approximately 40 to 150 x the LOD (as comparing to the Genome Equivalents/Swab values generated in the LOD with simulated clinical matrix study testing B/Malaysia/2506/2004 and B/Brisbane/60/2008, respectively). A polymorphism within segment PA of the Influenza B genome was identified at a position which is 4 nucleotides from the 3'-end of template 2. This G to A polymorphism results in a G/C (product/template) match to an A/C (product/template) mismatch. An A/C mismatch is determined to be moderately destabilizing, and coupled to its position only 4 nucleotides from the 3'-end of the template 2 recognition region, its impact on annealing is potentially great. The frequency of this G to A polymorphism is determined to be very low. In analyzing the strains present in the NCBI Influenza Virus Resource database from 2/2005 to 3/2014 (N=986), no strains contained this polymorphism, suggesting that it has not been circulating for an extended period of time.

[c] Influenza B/Wisconsin/01/2010 lowest level in which 3/3 replicates were positive is approximately 15 to 60 x the LOD, and Influenza B/Texas/06/2011 lowest level in which at least 1/3 replicates were positive is approximately 25 to 100 x the LOD (as comparing to the Genome Equivalents/Swab values generated in the LOD with simulated clinical matrix study testing B/Malaysia/2506/2004 and B/Brisbane/60/2008, respectively). A single G to A polymorphism within segment PA of the Influenza B genome was identified at a position which is 5 nucleotides from the 3'-end of the molecular beacon annealing region in both strains. The G to A polymorphism results in a C/G match to a C/A mismatch between the molecular beacon and product 1. The C/A mismatch is determined to be moderately destabilizing that can significantly reduce assay sensitivity. An assessment of what impact this polymorphism would have on the melting temperature (Tm) of the molecular beacon/product 1 annealing was performed and the results showed a Tm drop from 62.3°C to 55.6°C, just below the assay running temperature. This suggests that annealing would occur, but at a greatly reduced level, with a concomitant loss of assay sensitivity. The frequency of this G to A polymorphism is found at a frequency of approximately 5% within the NCBI Influenza Virus Resource database covering the time frame from 2/2005 through 3/2014.

An additional analytical reactivity study was also performed testing the same set of influenza A and B strains as described in the table above following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral

Transport Media, and demonstrated equivalent analytical reactivity performance to that of testing direct swab samples.

Analytical Specificity (Cross Reactivity)

To determine the analytical specificity of Alere™ i Influenza A & B, 53 commensal and pathogenic microorganisms (37 bacteria, 15 viruses and 1 yeast) that may be present in the nasal cavity or nasopharynx were tested. All of the following microorganisms were negative when tested at concentrations ranging from 10^8 to 10^{10} cells/mL, CFU/mL or IFU/mL (bacteria), 10^4 to 10^8 $TCID_{50}$/mL or $CEID_{50}$/mL (viruses), and 10^8 cells/mL (yeast).

Bacteria	**Viruses**	**Yeast**
Acinetobacter calcoaceticus	Adenovirus type 1	*Candida albicans*
Bacteroides fragilis	Adenovirus type 7	
Bordetella pertussis	Human Coronavirus OC43	
Chlamydia pneumoniae	Human Coronavirus 229E	
Corynebacterium diphtheriae	Enterovirus/Coxsackievirus B4	
Enterococcus faecalis	Human Cytomegalovirus (CMV) (Herpes V)	
Escherichia coli	Epstein Barr Virus	
Gardnerella vaginalis	Human metapneumovirus	
Haemophilus influenzae	Measles (Edmonston)	
Klebsiella pneumoniae	Mumps (Enders)	
Lactobacillus casei	Parainfluenza 1	
Lactobacillus plantarum	Parainfluenza 2	
Legionella pneumophila	Parainfluenza 3	
Listeria monocytogenes	Respiratory Syncytial Virus type B	
Moraxella/Branhamella catarrhalis	Rhinovirus type 1A	
Mycobacterium avium		
Mycobacterium intracellulare		
Mycobacterium tuberculosis		
Mycoplasma pneumoniae		
Neisseria gonorrhoeae		
Neisseria meningitidis		
Neisseria sicca		
Neisseria subflava		
Proteus vulgaris		
Pseudomonas aeruginosa		
Serratia marcescens		
Staphylococcus aureus		
Staphylococcus epidermidis		
Streptococcus, Group A		
Streptococcus, Group B		
Streptococcus, Group C		
Streptococcus, Group F		
Streptococcus, Group G		
Streptococcus mutans		
Streptococcus pneumoniae		
Streptococcus salivarius		
Streptococcus sanguinis		

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the nasal cavity or nasopharynx, were evaluated with Alere™ i Influenza A & B at the concentrations listed below and were found not to affect test performance.

Substance	Concentration
Mucin	20 µg/mL
Whole Blood	50 µl/mL
Sinus Buster Nasal Spray	200 µl/mL
NeoSynephrine Cold & Sinus Extra Strength Spray	200 µl/mL
Zicam Extreme Congestion Relief	200 µl/mL
Afrin PumpMist Original	200 µl/mL
4-acetamidophenol	200 µg/mL
Acetylsalicylic acid	650 µg/mL
Albuterol	400 ng/mL
Chlopheniramine	145 ng/mL
Dexamethasone	0.80 mg/mL
Dextromethorphan	1 µl/mL
Diphenhydramine	5 µg/mL
Doxylamine Succinate	236 ng/mL
Ephedrine	237 ng/mL
Flunisolide	6.8 ng/mL
Guaiacol glycerol ether	3.5 ng/mL
Mupirocin	12 mg/mL
Oxymetazoline	0.6 mg/mL
Phenylephrine	12 mg/mL
Rebetol	4.5 µg/mL
Relenza	282 ng/mL
Rimatadine	282 ng/mL
Tamiflu	1.1 µg/mL
Tobryamycin	2.43 mg/mL
Triamcinolone	40 µg/mL

Inhibition by other Microorganisms

Alere™ i Influenza A & B test performance in the presence of non-influenza respiratory pathogens was evaluated. Vendor provided stocks of influenza A and B strains were diluted in UTM to approximately 2 to 3 times the limit of detection. Contrived influenza A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. The following panel of non-influenza viruses were tested at the concentration provided in the table below and was found not to affect test performance.

Virus Panel	Concentration ($TCID_{50}$/ml)
Adenovirus Type 1	1.58×10^7
Rhinovirus Type 1A	1.58×10^7
Respiratory Syncytial Virus, Type B, Strain 18537	8.89×10^5

In an additional study, contrived influenza A and B positive swab specimens were also eluted into UTM and tested according the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media using

the same panel of non-influenza respiratory viruses as described in the table above. None of the non-influenza respiratory viruses tested was found to affect test performance at the concentrations evaluated.

Inhibition by High Levels of Influenza A and B

Alere™ i Influenza A & B test performance in the presence of high levels of influenza A and B was evaluated. Vendor provided stocks of influenza A and B strains were diluted in UTM to approximately 2 to 3 times the limit of detection. Contrived influenza A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. To create the co-infection swabs, diluted influenza A (at a concentration approximately 5 times the LOD) was added to the near LOD Flu B swab. Likewise, diluted influenza B (at a concentration approximately 40 times the LOD) was added to the near LOD Flu A swab. No impact on test performance was observed.

Alere™ i Influenza A & B test performance in the presence of high levels of influenza A and B was also evaluated in an additional study following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media. No impact on test performance was observed at the concentrations tested.

Carry-Over Contamination

An analytical carry-over study was performed to demonstrate that when recommended laboratory practices are followed, there is little risk of false positive results caused by carryover or cross-contamination in the Alere™ i Influenza A & B test. Vendor provided stocks of influenza A and B strains were diluted in UTM to a minimum of 10^6 $TCID_{50}$/ml. Contrived influenza A and B positive swab specimens were prepared by coating 10 microliters of virus dilution onto each swab. Testing of the contrived positive swabs was alternated with testing of a negative swab sample for a total of 15 rounds. There were no false positive results obtained.

An additional analytical carry-over study was performed testing contrived positive VTM samples alternated with negative VTM samples following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media for a total of 30 rounds. No false positive results were observed in this study except for one Flu B false positive result.

An additional analytical reactivity study was also performed testing the same set of influenza A and B strains as described in the table above following the test procedure for Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media, and demonstrated equivalent analytical reactivity performance to that of testing direct swab samples.

CLINICAL STUDY

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media

Clinical performance characteristics of Alere™ i Influenza A & B were evaluated in a multi-site prospective study during the 2014-2015 flu season in the U.S. A total of three investigational sites across the U.S. participated in the study. To be enrolled in the study, patients had to be presenting at the participating study centers with flu-like symptoms. Nasal or nasopharyngeal swab specimens were collected from patients with flu-like symptoms and were placed in viral transport media. The samples were processed and tested using the Alere™ i Influenza A & B assay according to the test procedure for **Nasal or Nasopharyngeal Swab Eluted in Viral Transport Media**. An FDA-cleared influenza real-time Polymerase Chain Reaction (RT-PCR) test was utilized as the comparator method for this study. All discrepant samples were tested on a different FDA-cleared influenza real-time RT-PCR assay at Alere Scarborough Inc. to confirm influenza status. External control testing, using Alere™ i Influenza A & B Positive and Negative Controls, was performed prior to sample testing each day and on each Alere™ i instrument for the duration of the clinical study. A total of 1,273 nasal or nasopharyngeal swabs eluted in viral transport media were enrolled in this study. Of those, 3 specimens did not meet eligibility criteria. A total of 1,270 viral transport media specimens were tested with the Alere™ i Influenza A & B assay. Patient age and gender distribution for all included specimens in this study is presented in the table below.

Age and Gender Distribution – Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media Study

Age Group	Female	Male
<1 year	92	84
1 to 5 years	258	282
6 to 10 years	113	131
11 to 15 years	63	56
16 to 21 years	16	20
>21 to 60 years	52	37
>60 years	42	24
Total	636	634

Of the 1,270 specimens, Alere™ i Influenza A & B generated invalid results for 27 specimens after repeat testing per the product instructions, resulting in a total of 1,243 specimens for performance analysis.

Compared to the comparator method, the performance of Alere™ i Influenza A & B for influenza A and influenza B are presented in the two tables below.

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media - Performance Obtained for Influenza A with Alere™ i Influenza A & B against the Comparator Method

Alere™ i Influenza A & B – Flu A	Comparator Method		
	Positive	Negative	Total
Positive	221	35[a]	256
Negative	5	982	987
Total	226	1017	1243
Sensitivity: 221/226	97.8% (95%CI: 94.9%-99.1%)		
Specificity: 982/91017	96.6% (95%CI: 95.3%-97.5%)		

[a] Flu A nucleic acid was detected in 31/35 False Positive specimens using an alternative FDA-cleared molecular test

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media - Performance Obtained for Influenza B with Alere™ i Influenza A & B against the Comparator Method

Alere™ i Influenza A & B – Flu B	Comparator Method		
	Positive	Negative	Total
Positive	92	19[a]	111
Negative	7	1125	1132
Total	99	1144	1243
Sensitivity: 92/99	92.9% (95%CI: 86.1%-96.5%)		
Specificity: 1125/1141	98.3% (95%CI: 97.4%-98.9%)		

[a] Flu B nucleic acid was detected in 3/19 False Positive specimens using an alternative FDA-cleared molecular test

Performance of Alere™ i Influenza A & B for the detection of influenza A and influenza B versus the comparator method in this study is presented in the table below stratified by patient age.

Nasal or Nasopharyngeal Swabs Eluted in Viral Transport Media - Performance Obtained for Influenza A and Influenza B with Alere™ i Influenza A & B in Comparison to the Comparator Method – Stratified by Patient Age

	≤ 5 Years of Age (n = 706)		6 - ≤ 21 Years of Age (n = 388)		≥ 22 Years of Age (n = 149)	
Influenza Type	**Sensitivity 95% CI**	**Specificity 95% CI**	**Sensitivity 95% CI**	**Specificity 95% CI**	**Sensitivity 95% CI**	**Specificity 95% CI**
Flu A	99.1% (105/106) 94.8%, 99.8%	98.2% (589/600) 96.7%, 99.0%	97.0% (98/101) 91.6%, 99.0%	95.8% (275/287) 92.8%, 97.6%	94.7% (18/19) 75.4%, 99.1%	90.8% (118/130) 84.6%, 94.6%
Flu B	100% (39/39) 91.0%, 100%	98.2% (655/667) 96.9%, 99.0%	94.2% (49/52) 84.4%, 98.0%	98.5% (331/336) 96.6%, 99.4%	50% (4/8) 21.5%, 78.5%	98.6% (139/141) 95.0%, 99.6%

During this prospective clinical study, the initial invalid rate (before repeat testing per the product instructions) was 4.3% (54/1270) (95% CI: 3.3% to 5.5%). After repeat testing per the product instructions, the invalid rate was 2.1% (27/1270) (95% CI: 1.5%, 3.1%).